American Journal of Medical Genetics 65:149–154 (1996)

Unusual Xanthomas in a Young Patient With Heterozygous Familial Hypercholesterolemia and Type III Hyperlipoproteinemia

Giso Feussner, Jürgen Dobmeyer, Henrik Nissen, and Torben Stiig Hansen

Department of Internal Medicine I, Endocrinology and Metabolism, University of Heidelberg, Heidelberg, Germany (G.F., J.D.); and Department of Clinical Chemistry, Odense University Hospital, Odense C, Denmark (H.N., T.S.H.)

We report on a 20-year-old man with the combination of two independent familial lipoprotein disorders: heterozygous familial hypercholesterolemia (FH) and type III hyperlipoproteinemia (HLP). Familial hypercholesterolemia was diagnosed by elevated total and low density lipoprotein cholesterol levels and family history. By denaturing gradient gel electrophoresis, DNA sequencing and restriction fragment length polymorphism analysis, a G→A splice donor mutation in intron 3 of the proband's low density lipoprotein receptor gene was identified as the underlying molecular defect. This mutation was described previously as a receptor-negative founder mutation in Norway (FH-Elverum) and subsequently in 6 unrelated heterozygous English patients, creating a severe phenotype of familial hypercholesterolemia. Type III HLP was confirmed by homozygosity for apolipoprotein (apo) E2 and an elevated ratio of very low density lipoprotein cholesterol to serum triglycerides (0.40; normal ratio about 0.20). The patient has unusual flat xanthomas in the interdigital webs of the hands which are normally not found in either disease. These dermatological findings might therefore be indicative of the rare combination of both disorders of lipoprotein metabolism in one individual. © 1996 Wiley-Liss, Inc.

Received for publication September 2, 1994; revision received October 13, 1995.

Address reprint requests to G. Feussner, M.D., Department of Internal Medicine I, Endocrinology and Metabolism, University of Heidelberg, Bergheimer Strasse 58, 69115 Heidelberg, Germany.

Abbreviations: Apo, apolipoprotein; DGGE, denaturing gradient gel electrophoresis; FH, familial hypercholesterolemia; HDL, high density lipoproteins; HLP, hyperlipoproteinemia; HMG CoA, hydroxy-methyl-glutaryl coenzyme A; IDL, intermediate density lipoproteins; IEF, isoelectric focusing; LDL, low density lipoproteins; RFLP, restriction fragment length polymorphism; VLDL, very low density lipoproteins.

KEY WORDS: apolipoprotein E, familial dysbetalipoproteinemia, familial hypercholesterolemia, type III hyperlipoproteinemia, xanthomas

INTRODUCTION

Familial hypercholesterolemia (FH) is an autosomal dominant disorder of defective low density lipoprotein (LDL) receptor function with an estimated heterozygote prevalence of about 1:500 in the general population [Goldstein and Brown, 1989]. Heterozygous FH is associated with approximately twofold increased LDL cholesterol levels and increased risk for premature coronary heart disease [Goldstein and Brown, 1989]. Typical dermatological findings are xanthelasmas, tubero-eruptive xanthomas at the elbows and knees and tendinous xanthomas of the Achilles tendons and/or the tendons of the hand. However, FH is a rather heterogeneous disease and until now more than 150 different mutant alleles at the LDL receptor gene locus with a variable clinical phenotype have been identified [Hobbs et al., 1992]. In contrast, type III hyperlipoproteinemia (HLP) is a more homogeneous genetic disorder. Type III HLP is a disturbance of the metabolism of triglyceride (TG)- and cholesterol-rich remnants of chylomicrons, very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), collectively referred to as β-VLDL. Affected individuals develop accelerated atherosclerosis involving both coronary and peripheral arteries [Brewer et al., 1983; Mahley and Rall, 1989; Feussner et al., 1993a,b]. Typical findings are palmar xanthomas (orange-yellowish discolorations of the palmar creases) which have been described to occur in about 39% of patients [Feussner et al., 1993a]. The most common underlying genetic defect for the lipoprotein abnormalities in type III HLP is the synthesis of an abnormal isoform of apolipoprotein (apo) E, apo E2, which does not bind normally to lipoprotein receptors [Schneider et al., 1981]. Approximately 1% of the general population are homozygous for apo E2, but only about 2% of these genetically predisposed individuals finally develop type III HLP at some stage of their lives. Therefore, the

prevalence of this lipoprotein disorder is estimated to be around 1:5,000 in the general population. We are aware of only 3 reports describing the combination of FH and type III HLP in 4 patients [Hazzard et al., 1981; Nestel et al., 1984; Sakumara et al., 1995]. The underlying mutations at the LDL receptor gene locus in these subjects have not been reported. Flat xanthomas in the interdigital webs of the hands are present in 3 of the patients. However, these skin alterations are not found in "classical" type III HLP or FH. Here we describe a 20-year-old apo E2 homozygous man with heterozygous FH with similar dermatological findings. Hence, flat xanthomas in the interdigital webs might be indicative of the combination of these independent disorders of lipoprotein metabolism.

CLINICAL REPORT

Index Case

The index case (W.H.) was a 20-year-old, slightly overweight (77 kg, 175 cm, body mass index [BMI] 25.1 kg/m^2) German man. Hyperlipidemia and xanthomas were noted for the first time at age 19. At that time total cholesterol was reported to be elevated between 400 and 500 mg/dl and lipid-lowering therapy with colestipol (20 g/day) was instituted by the patient's family doctor. Tubero-eruptive xanthomas at the elbows and slightly eruptive xanthomas in the palmar creases (xanthoma striatum palmare) were described. When the patient was referred to our hospital, about 6 months later, we found small eruptive xanthomas at his elbows, xanthomas in the palmar creases (Fig. 1A), tubero-eruptive xanthomas on the proximal interphalangeal joints of the fingers and unusual flat yellow-orange xanthomas in the interdigital webs of the hands (Fig. 1B). Xanthomas of the knees or thickening of the Achilles tendons were not observed. Initial lipoprotein analysis (under therapy with 20 g colestipol per day) showed a total cholesterol of 438, HDL-cholesterol 34, LDL-cholesterol 340, VLDL-cholesterol 63, and triglycerides of 159 mg/dl, respectively (II-3 in Table I). The VLDL-cholesterol-to-serum triglyceride ratio was elevated at 0.40 (normal ratio about 0.20). Homozygosity for apo E2 was demonstrated by isoelectric focusing (IEF) according to Warnick et al. [1979]. All other analysed blood parameters were normal and there was no indication for secondary hyperlipidemia. The patient was otherwise healthy and inconspicuous. Currently there are no symptoms of premature or accelerated atherosclerosis as examined by exercise test and Doppler ultrasonography of the carotid and peripheral arteries of the legs.

Relatives

Lipid data of the analysed relatives are given in Table I. The mother (I-1) has elevated total and LDL-cholesterol and the heterozygous apo E3/2 phenotype. The father (I-2) is also apo E3/2 heterozygous, but normolipidemic. The 11-year-old sister (II-1) is apo E3/3 homozygous. However, her total and LDL-cholesterol values are distinctly elevated. The 17-year-old brother (II-2) has rather low total and LDL-cholesterol concentrations and the homozygous apo E2/2 phenotype (dysbetalipoproteinemia). The maternal grandmother has had coronary artery disease and aortocoronary bypass surgery was performed. She died of myocardial infarction at age 62. The other relatives were not informative. The pedigree of the analysed family is given in Figure 2.

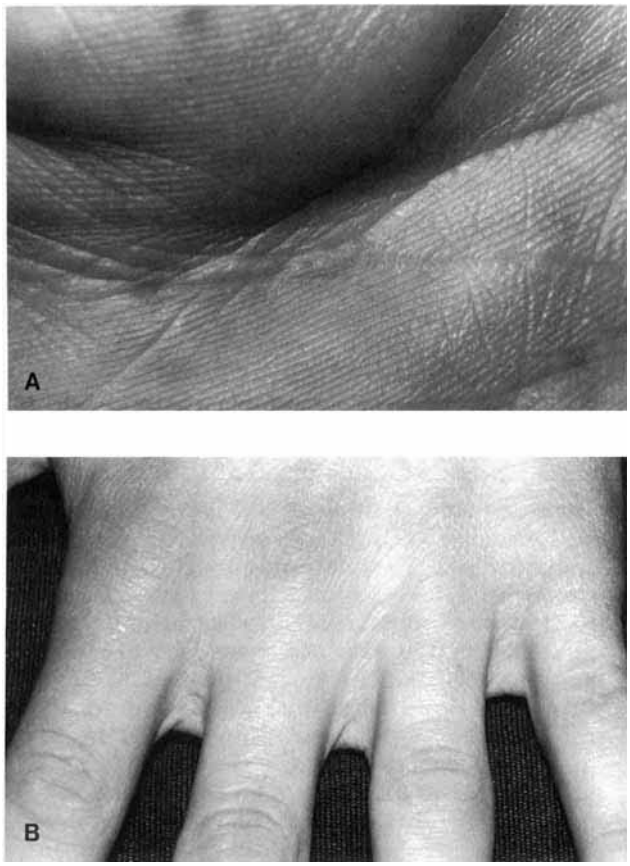

Fig. 1. Eruptive xanthomas in the palmar creases of the hand (xanthoma striatum palmare; **A**), and unusual flat yellow-orange xanthomas in the interdigital webs of the hand and tubero-eruptive xanthomas on the proximal interphalangeal joints of the fingers (**B**) of the index patient (W.H.) with heterozygous FH and type III HLP.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE analysis of the LDL receptor gene promotor region, the 18 LDL receptor gene exons, including at least 5 intron bases on both sides of the individual exons, and of the codon 3456–3553 region, was performed exactly as described recently [Nissen et al., 1996]. In short, each of the DNA regions was amplified using polymerase chain reaction (PCR) primer pairs, where one primer in each pair had a 40–50 bp GC-clamp attached. The GC-clamped PCR products were then loaded onto 6% polyacrylamide gels containing either a 30–70% or a 40–80% denaturing gradient gel (100% denaturant = 7 M urea and 40% formamide), electrophoresed for 5 h at 150 V and 60°C, stained in ethidium bromide, and photographed under ultraviolet translumination.

Sequencing

Exon 3 of the LDL receptor gene of the propositus (W.H.) was PCR-amplified using the exon 3 DGGE PCR

TABLE I. Lipid Data in the W.H. Family*

Number in the pedigree	TG	Chol.	HDL-C	LDL-C	VLDL-C	VLDL-C	Apo E phenotype	Het. FH
	(mg/dl)					Serum TG		
I-1	137	379	38	318	24	0.18	3/2	+
I-2	141	200	41	133	26	0.18	3/2	−
II-1	113	382	39	326	17	0.15	3/3	+
II-2	137	153	44	75	34	0.25	2/2	−
II-3	159	438	34	340	63	0.40	2/2	+

* TG, triglycerides; Chol., cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; Het. FH, heterozygous familial hypercholesterolemia.

primers in non-GC clamped editions and sequenced using standard solid phase sequencing of single-stranded Dynabead (Dynal, Oslo, Norway) isolated PCR templates with automated sequence detection equipment (ALF, Pharmacia, Uppsala, Sweden).

Restriction Fragment Length Polymorphism (RFLP) Analysis

The detected mutation in the patient's 268 bp LDL receptor exon 3 fragment introduces an MslI site giving fragments of 222 and 46 bp lengths. The sequencing results were verified by digestion of 10 μl of the exon 3 DGGE PCR product with MslI. The LDL receptor gene exon 2 SfaNI polymorphism [Soutar, 1992] was analysed by SfaNI digestion. Both MslI and SfaNI digests were done under conditions as recommended by the manufacturer (New England Biolabs, Beverly, MA). The digests were run on a 3% agarose gel, stained in ethidium bromide and photographed under ultraviolet translumination.

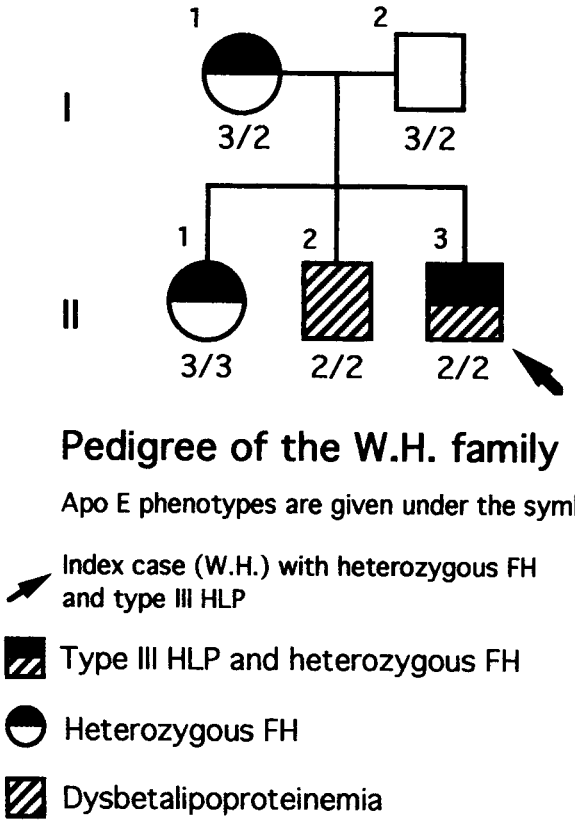

Fig. 2. Pedigree of the W.H. family. The index patient (W.H.) is marked by the arrow.

RESULTS

The clinical and laboratory findings as well as the family history of W.H. are compatible with the diagnosis of heterozygous FH (type IIa hyperlipoproteinemia). DGGE analysis of the LDL receptor gene promotor region, the 18 LDL receptor gene exons, and of the codon 3456–3553 region resulted in the appearance of an abnormal 4-band pattern in exons 2 and 3 of the propositus. These pattern types in a DGGE analysis indicate the presence of a sequence variation between the two alleles in the LDL receptor gene exon 2 and 3 regions. The pattern in exon 2 looked like heterozygosity for the LDL receptor gene SfaNI polymorphism [Soutar, 1992], and this in turn was verified by enzyme digestion (data not shown). No polymorphisms in exon 3 of the LDL receptor gene are known, and as the exon 3 DGGE pattern (Fig. 3) cosegregated in the family with clinically overt FH, exon 3 (including the adjacent intron-exon boundaries) was sequenced in the propositus. Sequencing showed a heterozygous G→A mutation in the first base of intron 3, thus destroying the conserved GT splice donor site of intron 3. This mutation has originally been described by Leren et al. [1994] as FH-Elverum. The mutation introduces an MslI site and digestion with this enzyme subsequently verified the sequencing results (Fig. 4).

The VLDL-cholesterol-to-serum triglyceride ratio in the patient at 0.40 was elevated, which is unusual for FH (normal ratio about 0.20), demonstrating delayed catabolism of triglyceride- and cholesterol-rich VLDL-particles. Therefore, the coexistence of type III HLP was postulated and proved by IEF showing homozygosity for apo E2. Thus, the severe hyperlipidemia of the patient is explained by the presence of two independent familial lipoprotein disorders (heterozygous FH and type III HLP). Familial defective apolipoprotein B-100, a ligand defect leading to hypercholesterolemia, was definitively excluded in the patient by DGGE analysis [Nissen et al., 1995]. Lipid-lowering therapy with the resin colestipol was increased to a maximum dose of 30 g per day. As total and LDL-cholesterol concentrations were only moderately reduced by this regimen, in addi-

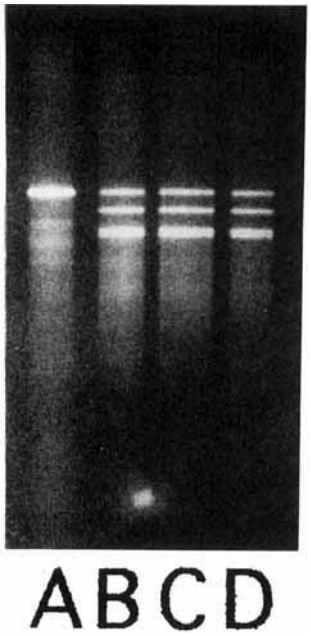

Fig. 3. DGGE examination of the exon 3 region of the human LDL receptor gene in the W.H. family. **Lane A:** Healthy father, I-2; **lane B:** affected mother, I-1, with heterozygous FH; **lane C:** affected sister, II-1, with heterozygous FH; **lane D:** index patient, II-3, with heterozygous FH and type III HLP. The healthy brother, II-2, of the propositus was not available for the analysis.

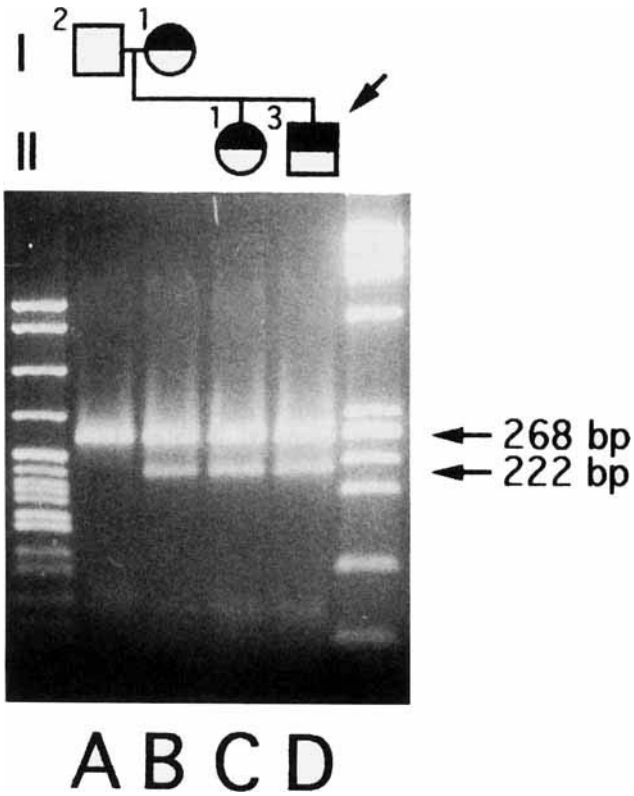

Fig. 4. RFLP analysis with MslI of the 268 bp LDL receptor gene exon 3 PCR fragment (covering LDL receptor exon 3 [123 base pairs] and adjoining intron 2 [55 base pairs] and intron 3 [50 base pairs] sequences) in the W.H. family. In healthy individuals the 268 bp PCR fragment is not cleaved by MslI. In affected individuals with the G→A splice donor mutation in intron 3 (FH-Elverum), a new recognition site for the endonuclease MslI is created, leading to two fragments of 222 and 46 bp in length. FH-Elverum hetrozygotes are identified therefore by a two-band pattern (note that the 46 bp fragment was not visible on electrophoresis). **Lane A:** Healthy father, I-2; **lane B:** affected mother, I-1, with heterozygous FH; **lane C:** affected sister, II-1, with heterozygous FH; **lane D:** index patient with heterozygous FH and type III HLP. The healthy brother, II-2, of the propositus was not available for the analysis. The index patient (W.H.) is marked by the arrow. The lettering of the symbols is exactly as in Figure 2.

tion simvastatin in a daily dose of 20 mg, respectively 40 mg, was given after 6, respectively 9, months of therapy. The hydroxy-methyl-glutaryl-coenzyme A (HMG CoA) reductase inhibitor simvastatin is known to be efficient in the therapy of heterozygous FH [Grundy, 1988] and type III HLP [Stuyt et al., 1990, 1991; Feussner et al., 1992]. Combination therapy of both drugs reduced the initially severely elevated total and LDL-cholesterol concentrations by about 43% (Fig. 5). In addition, within 2 years of treatment, there has been a complete regression of the palmar xanthomas, the tubero-eruptive xanthomas at the elbows and on the proximal interphalangeal joints of the fingers and a marked reduction of the flat xanthomas in the interdigital webs of the hands. Therefore, this therapy proved to be effective not only in reducing elevated cholesterol levels but also in the regression of cutaneous lipid depositions.

DISCUSSION

This is a report of the unusual combination of heterozygous FH with type III HLP in one subject. The prevalence of heterozygous FH is about 1:500 [Goldstein and Brown, 1989] and homozygosity for apo E2 is approximately 1:100 [Davignon et al., 1988]. Therefore, the combination of both defects is estimated to be around 1:50,000 in the general population. However, only 4 individuals have been described [Hazzard et al., 1981; Nestel et al., 1984; Sakuma et al., 1995] with the concurrence of these two genetically independent errors of metabolism. The respective mutations at the LDL receptor gene locus in these subjects have not been disclosed. However, the molecular defect in our patient was FH-Elverum [Leren et al., 1994], which was described first as a founder mutation in Norway. The mutation affects the first base of the GT donor splice site consensus sequence in intron 3 and has so far not been reported in Germany, but a detailed study of the mutation detected in 6 unrelated English patients with heterozygous FH was published recently [Sun et al., 1995]. Here, it was found that the FH-Elverum mutation resulted in complete skipping of exon 3 during mRNA processing, thereby giving an LDL receptor protein lacking repeat 2 of the binding domain. In addition, the mutation resulted in inefficient splicing with reduced mutant mRNA levels and, consequently, reduced total LDL receptor protein level, affecting not only LDL affinity, but also LDL receptor numbers. The mutation obviously has far reaching implications, since FH-Elverum homozygotes have been shown to be receptor negative [Leren et al., 1994]. These findings are consis-

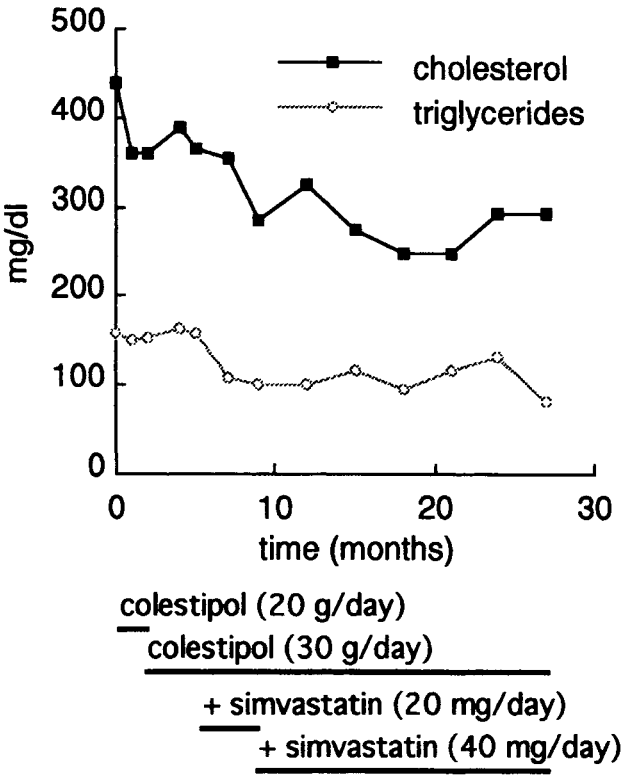

Fig. 5. Time course of total cholesterol and triglyceride concentrations under lipid-lowering therapy of the index patient (W.H.) with heterozygous FH and type III HLP.

tent with the clinical phenotype, leading to severe xanthomatosis and distinctly elevated serum cholesterol [Leren et al.,1994; Sun et al., 1995].

Surprisingly, 4 of the 5 patients with the combination of FH and type III HLP (including the one reported here) had unusual flat xanthomas in the interdigital webs of the hands, which are not found in either disease. Therefore, this dermatological finding might be typical and indicative of the combination of both disorders.

Type III HLP has rarely been reported in children and young adults. Till now, there have only been 10 reports of pediatric or adolescent patients with the disease [Godolphin et al., 1972; Mishkel et al., 1975; Glueck et al., 1976; Hazzard et al., 1981; Havel et al., 1983; Nestel et al., 1984; Lindner and Illingworth, 1988; Mabuchi et al., 1989; Rall et al., 1989; Feussner et al., 1990]. The presence of heterozygous FH together with homozygosity for apo E2, leading to VLDL-remnant accumulation, may be responsible for the exacerbation of the severe hyperlipidemia in this individual and the early (and unusual) clinical manifestation of type III HLP. Serum and LDL-cholesterol concentrations could be reduced satisfactorily (but not normalized) by combined resin/HMG CoA reductase inhibitor therapy. In addition, a remarkable regression of the xanthomas, within 2 years of treatment, was observed. Therefore, not only patients with heterozygous FH, but also individuals with the combination of FH and type III HLP seem to benefit from this intensified lipid-lowering regimen.

In summary, this is the fourth report of the combination of heterozygous FH and type III HLP in one individual and the first description of the underlying molecular defect at the LDL receptor gene locus. Four of the 5 reported cases had unusual flat xanthomas in the interdigital webs of the hands. Therefore, this clinical syndrome might be typical and indicative of the coexistence of both disorders of lipoprotein metabolism.

REFERENCES

Brewer HB Jr, Zech LA, Gregg RE, Schwartz D, Schaefer EJ (1983): Type III hyperlipoproteinemia: Diagnosis, molecular defects, pathology, and treatment. Ann Intern Med 98:623–640.

Davignon J, Gregg RE, Sing CF (1988): Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8:1–21.

Feussner G, Wingen AM, Ziegler R (1990): Type III hyperlipoproteinemia in a child with hemolytic uremic syndrome. Metabolism 39:1196–1199.

Feussner G, Eichinger M, Ziegler R (1992): The influence of simvastatin alone and in combination with gemfibrozil on plasma lipids and lipoproteins in patients with type III hyperlipoproteinemia. Clin Investig 70:1027–1035.

Feussner G, Wagner A, Kohl B, Ziegler R (1993a): Clinical features of type III hyperlipoproteinemia: Analysis of 64 patients. Clin Investig 71:362–366.

Feussner G, Wagner A, Ziegler R (1993b): Relation of cardiovascular risk factors to atherosclerosis in type III hyperlipoproteinemia. Hum Genet 92:122–126.

Glueck CJ, Fallat RW, Mellis MJ, Steiner PM (1976): Pediatric familial type III hyperlipoproteinemia. Metabolism 25:1269–1274.

Godolphin WJ, Conradi G, Campbell DJ (1972): Type-III hyperlipoproteinemia in a child. Lancet 1:209–210.

Goldstein JL, Brown MS (1989): Familial hypercholesterolemia. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): "The Metabolic Basis of Inherited Disease," 6th ed. New York: McGraw-Hill Book Co., pp 1215–1250.

Grundy SC (1988): HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. N Engl J Med 319:24–33.

Havel RJ, Kotite L, Kane JP, Tun P, Bersot T (1983): Atypical familial dysbetalipoproteinemia associated with apolipoprotein phenotype E3/3. J Clin Invest 72:379–387.

Hazzard WR, Miller N, Albers JJ, Warnick GR, Baron P, Lewis B (1981): Association of isoapolipoprotein-E3 deficiency with heterozygous familial hypercholesterolaemia: Implications for lipoprotein physiology. Lancet 1:298–301.

Hobbs HH, Brown MS, Goldstein JL (1992): Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat 1:445–466.

Leren TP, Solberg K, Rodningen OK, Tonstad S, Ose L (1994): Two founder mutations in the LDL receptor gene in Norwegian familial hypercholesterolemia subjects. Atherosclerosis 111:175–182.

Lindner MA, Illingworth DR (1988): Expression of type III hyperlipoproteinemia in an adolescent patient with hypothyroidism. J Pediatr 113:86–89.

Mabuchi H, Itoh H, Takeda M, Kajinami K, Wakasugi T, Koizumi J, Takeda R, Asagami C (1989): A young type III hyperlipoproteinemic patient associated with apolipoprotein E deficiency. Metabolism 38:115–119.

Mahley RW, Rall SC, Jr (1989): Type III hyperlipoproteinemia (dysbetalipoproteinemia): The role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): "The Metabolic Basis of Inherited Disease," 6th ed. New York: McGraw-Hill Book Co., pp 1195–1213.

Mishkel MA, Nazir DJ, Crowther S (1975): A longitudinal assessment of lipid ratios in the diagnosis of type III hyperlipoproteinemia. Clin Chim Acta 58:121–136.

Nestel PJ, Reardon MF, Fidge NH (1984): Homozygous familial hypercholesterolemia occurring with apolipoprotein E3 deficiency. Report of two cases. Arteriosclerosis 4:124–129.

Nissen H, Guldberg P, Hansen AB, Petersen NE, Horder M (1996): Clinically applicable mutation screening in familial hypercholesterolemia. Hum Mutat, in press.

Nissen H, Hansen PS, Faergeman O, Horder M (1995): Mutant screening of the codon 3500 region of the apolipoprotein B gene by denaturing gradient-gel electrophoresis. Clin Chem 41:419–423.

Rall SC Jr, Newhouse YM, Clarke HRG, Weisgraber KH, McCarthy BJ, Mahley RW, Bersot TP (1989): Type III hyperlipoproteinemia associated with apolipoprotein phenotype E3/3: Structure and genetics of a rare apolipoprotein E3 variant. J Clin Invest 83: 1095–1101.

Sakuma N, Iwata S, Ikeuchi R, Ichikawa T, Hibino T, Kamiya Y, Ohte N, Kawaguchi M, Junimatsu M, Kawahara H, Fujinami T (1995): Coexisting type III hyperlipoproteinemia and familial hypercholesterolemia: A case report. Metabolism 44:460–465.

Schneider WJ, Kovanen PT, Brown MS, Goldstein JL, Utermann G, Weber W, Havel RJ, Kotite L, Kane JP, Innerarity TL, Mahley RW (1981): Familial dysbetalipoproteinemia: Abnormal binding of mutant apolipoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenals of rats, rabbits and cows. J Clin Invest 68:1075–1085.

Soutar AK (1992): A polymorphism in exon 2 of the human LDL-receptor gene (LDLR). Nucleic Acids Res 19:4314.

Stuyt PMJ, Mol MJTM, Stalenhoef AFH, Demacker PNM, van't Laar A (1990): Simvastatin in the effective reduction of plasma lipoprotein levels in familial dysbetalipoproteinemia (type III hyperlipoproteinemia). Am J Med 88:42N–45N.

Stuyt PMN, Mol MJTM, Stalenhoef AFH (1991): Long-term effects of simvastatin in familial dysbetalipoproteinaemia. J Intern Med 230:151–155.

Sun X-M, Patel DD, Bhatnagar D, Knight BL, Soutar AK (1995): Characterization of a splice-site mutation in the gene for the LDL receptor associated with an unpredictably severe clinical phenotype in English patients with heterozygous FH. Arterioscler Thromb Vasc Biol 15:219–227.

Warnick GR, Mayfield C, Albers JJ, Hazzard WR (1979): Gel isoelectric focusing method for specific diagnosis of familial hyperlipoproteinemia type 3. Clin Chem 25:279–284.